UCRL-JC-122299 Abs

Effect of subsurface electrical heating and steam injection on the indigenous microbial community

Paula Krauter, Don MacQueen, Dorothy Bishop
Lawrence Livermore National Laboratory

Abstract

Since the potential for contaminant bioremediation in steam treated subsurface environments has not been explored, the thermal remedial treatment of a gasoline spill at Lawrence Livermore National Laboratory's (LLNL) Livermore site provided an opportunity to study microbial community changes in the subsurface environment. Many terrestrial microorganisms die or become metabolically inactive if heated for a sufficient time at temperatures of 62-100°C thus thermal remediation techniques are expected to significantly alter the microbial community structure. We studied changes in community structure and population abundance as well as the characteristics of indigenous heat-tolerant microorganisms before and after steam treatment. Using fatty acid profiles from culturable microorganisms obtained from sediment cores before and after thermal treatment, a 90-98% decline in total microorganism populations in hot subsurface sediments (up to 94°C) was found. Surviving heat-tolerant microorganisms were found to possess elevated concentrations of saturated fatty acids in their lipid membranes. We also observed that some heat-tolerant microorganisms were capable of degrading gasoline compounds.

Introduction

Underground spills of fuel hydrocarbons are difficult to remediate when the contaminants are contained in both the saturated and unsaturated zones and reside in relatively impermeable clays. The fuel spill at LLNL Livermore site was chosen as a site to test the Dynamic Underground Stripping (DUS) thermal remediation technique because hydrocarbons were present under such conditions. The DUS process employs electrical energy to heat clay and fine-grained sediments thereby vaporizing water and contaminants trapped within the soils, and forcing the contaminants into the steam-swept zones (Buettner and Daily, 1994). The steam is injected into wells, heating the contaminated sediments to approximately 100°C, then both contaminants and water are removed by vacuum extraction (Udell et. al., 1991). The first steam pass at the LLNL main site took place in February, 1993. Seventeen hundred gals of gasoline were subsequently removed. The second steam pass, extending several weeks during May through July, 1993, removed 4,600 gals of gasoline (Yow et al., 1995a; Yow et al., 1995b). Volumes were calculated from daily vapor and aqueous stream analyses. Sediment samples obtained 4 weeks after heat and steam treatments had core temperatures up to 94°C. Two years (September, 1995) after the heat and steam treatment the temperature of the ground water remained between 45 and 75°C.

Methods

Pairs of sediment cores (designated HW-GP-104 and GSB-807; and HW-GP-103 and TEP-GP-007) were investigated for the presence of microorganisms. Each pair consisted of one set of core samples collected from the gasoline contaminated area

before thermal treatment and one set collected about four weeks after the region was remediated by the DUS process. Although we attempted to locate the drilling sites for cores obtained after DUS treatment as close as possible to sample sites studied prior to DUS, well placements were largely based on engineering considerations.

Only aerobic microorganisms were examined since site-wide subsurface geochemistry indicates an aerobic subsurface environment (McNab and Narasimhan, 1994). In addition, data from oxygen vapor mass spectrometric analysis of soil cores collected prior to DUS suggests an aerobic vadose zone (Camp, 1992), and the DUS process added air to the subsurface through the injection of oxygenated steam. Sediment samples from the center of the gasoline plume before and after steam were analyzed for direct total cell counts and heterotrophic plate counts. The total numbers of microorganisms were assessed within cores using epifluorescence microscopy. Isolates were obtained from the heterotrophic plates and fatty acid methyl ester analysis was conducted on each morphologically distinct colony. Pure bacterial or fungal cultures were grown for 24 h on either trypticase soy agar, SAB or R2A (Reasoner and Geldreich, 1985); then fatty acids were extracted and methylated to produce fatty acid methyl esters for gas liquid chromatography (Miller and Berger, 1985) by Acculab Inc., Newark, Delaware.

Benzene, toluene, and BTEX degradation was measured by an HP 5890 gas chromatograph (GC) outfitted with a photoionization detector. Shake-flasks containing ground water and the microbial cultures were amended with 5 mg/L benzene or 5 mg/L toluene, or 25 mg/L of BTEX. BTEX degradation was estimated quantitatively by comparing the total peak area in the GC profile of the compound in the test solution with that of the control. To minimize quantitative errors the external standardization method was used. Triplicate analyses of the samples reproducible to within ±5% were considered.

Results

Microbial populations

The direct count population means from two centrally located cores, GSB-807 (sediment temperatures were about 18°C before DUS) and HW-GP-104 (collected after DUS temperatures reached 96°C) had a two order of magnitude difference between before (2.30×10^6 AODC/g dry wt sediment) and after (7.10×10^4 AODC/g dry wt sediment, $p=0.0001$, ANOVA) DUS treatment. Prior to the injection of heat and steam the microbial population size ranged from 1.5×10^6 to 4.6×10^6 AODC/g dry wt sediment throughout the treatment site; after the treatment, the population ranged from 3.3×10^4 to 4.3×10^5 AODC/g dry wt sediment. The greatest decline in cell numbers occurred in the localized area surrounding the steam vents (about 78 to 150 ft below ground surface).

Heterotrophic culturable microbial counts coordinately declined in thermally treated soils. Sediments obtained from the contaminated zone before (GSB-807) and after (HW-GP-104) thermal remediation decreased from a cumulative average of 1.10×10^4 to 2.12×10^3 CFU/gdw. Sediment samples from the underlying saturated zones of these two cores also decreased from a cumulative average of 4.74×10^3 to 9.28×10^2 CFU/gdw. Overall statistical analysis of the culturable heterotrophic microorganisms resulted in a significant difference between the before and after treatment heterotrophic microorganism counts ($p=0.026$, ANOVA), reflecting a two order of magnitude difference, similar to that found using total direct counts.

Community structure

We used the Microbial Identification System (MIDI) for phylogenetic classification of culturable microorganisms. The MIDI system matched similarity indices of the fatty acid profiles of isolated microorganisms to profiles of known organisms contained in the MIDI data base. Prior to DUS treatment, aerobic microorganisms isolated from TEP-GP-007 included three genera of fungi and seven genera of bacteria, plus some bacteria unidentifiable by comparison with the MIDI database. Both gram-positive and gram-negative microorganisms were isolated from these sediment samples. Gram-staining generally divides bacteria into two groups which differ in their cell wall structures. We anticipated minimal survival of the gram-negative microorganisms after heat treatment due to the low melting point of their major membrane fatty acids. This prediction was realized; all post-DUS treatment sediment isolates from HW-GP-103 (sediment sample depths were 111 and 115 ft below surface) were mostly gram-positive, spore forming microorganisms.

Sediments from TEP-GP-007 were contaminated with fuel hydrocarbons (ranging from 14 to 500 mg/kg sediment) before DUS. Organisms capable of degrading aromatic compounds, *Pseudomonas* and *Aspergillus*, were among those identified from these sediment samples. Organisms in the genera *Pseudomonas, Achromobacter, Bacillus, Arthrobacter, Corynebacterium, Norcardia, Mycobacterium, Penicillium, Aspergillus and Fusarium* have been identified as aromatic ring degraders (Krulwich and Pelliccione, 1979). Microorganisms isolated after thermal remediation were various gram-positive cocci species; no gram-negative species were recovered from these thermally treated sediments.

Lipid membrane biochemistry in heat-tolerant microorganisms

The predominant fatty acid composition detected in the group of culturable microorganisms was altered after thermal treatment. Prior to DUS treatment, microbial isolates contained a substantial percentage of monounsaturated fatty acids with *cis* stereochemistry (62.5%). Unsaturation, particularly with double bonds in *cis* stereochemistry, lowers the melting point of fatty acids. Because the temperature of transition in fatty acid composition increases if the hydrocarbon chains are straight and fully saturated, we anticipated that microorganisms containing saturated fatty acids would have greater survivability following DUS treatment. Table 2 illustrates the differences in distribution of fatty acid content of microorganisms isolated before and after DUS treatment. The Kolmogorov-Smirnov test for differences in distribution of fatty acid content before and after thermal treatment was statistically significant for all 5 examined fatty acids, supporting the observation that high concentrations of these 5 fatty acids occur in microorganisms isolated after DUS treatment. High proportions of saturated lipids in membranes or cell walls are often associated with thermophilic microorganisms. Most likely, organisms that survived DUS treatment originally contained higher concentrations of saturated fatty acids, rather than altering their ratio of unsaturated to saturated fatty acids. Kaneda (1991) states that very few organisms are capable of removing a double bond by hydrogenation of intact membrane lipids.

Gasoline utilization of heat-tolerant microorganisms

Heat-tolerant subsurface microorganisms may be potentially useful for cleaning up residual gasoline after mechanical thermal remedial schemes have been employed. Several species of microorganisms obtained from DUS remediated sediments were

tested for their ability to degrade fuel hydrocarbons. These isolates were capable of degrading gasoline compounds under the test conditions (Table 2).

Dynamic Stripping remediation was a strong selective force on the thermal survival of subsurface biota, however, a portion of the original microbial community survived the DUS treatment and several heat-tolerant isolates degraded gasoline compounds. Our results suggest that consideration should be given to biodegradation of residual fuel hydrocarbons in thermally treated sites by native surviving organisms. Consequently, it is important to understand the effect of a remediation process on indigenous microorganisms so that we may enhance conditions for future bioremediation activity.

Summary of Key results:

1. Total microbial populations decreased (90-98%) in the area of the steam injection wells following DUS treatment.
2. Total microbial populations decreased to a lesser extent (varying between 20 and 85%) in sediment core samples from the periphery of the thermal treatment zone.
3. Heat-tolerant microorganisms had significantly elevated concentrations of saturated fatty acids (*i*15:0, *a*15:0, *i*16:0, *i*17:0, and *a*17:0).
4. Despite the apparent microbial community structure change after DUS treatment, several surviving microorganisms were capable of degrading gasoline.

Acknowledgments

This work was funded by the Office of Technology Development, ADS No. 1540-02 and the U. S. Department of Energy under contract No. W-7405-Eng-48.

References

Buettner, H. M. and W. Daily (1994), Cleaning Contaminated Soil Using Electrical Heating and Air Stripping. *J. Environ Engineering.*

Camp, D. W. (1992), Soil gas composition provides evidence of *in situ* biodegradation of organic contaminants. Proceedings of 1992 HAZMACON Conference held 3/30 to 4/2/92 in Long Beach, California.

Kaneda, T. (1991), Iso- and anteiso-fatty acids in bacteria: biosynthesis, function, and taxonomic significance. *Microbiol. Rev*. **55**:288-302.

Krulwich, T. A. and N. J. Pelliccione (1979), *Ann. Revs. Microbiol*. **33**:95.

McNab, W. W. and T. N. Narasimhan (1994), Degradation of chlorinated hydrocarbons and ground water geochemistry: A field study. *Environ. Sci. and Technol*. **28**:769-775.

Miller, L. T. and T. Berger (1985), Bacteria identification by gas chromatography of whole cell fatty acids. Hewlett-Packard Application Note 228-241.

Reasoner, D. J., and E. E. Geldreich (1985), A New Medium for the Enumeration and Subculture of Bacteria from Potable Water. *Appl Environ Microbiol*. **49**:1-7.

Udell, K. S. Sitar, N., Hunt, J. R., Stewart, L. D. Jr. (1991), Process for in situ decontamination of subsurface soil and ground water, United States Patent 5,018,576.

Yow, J. L., Jr., R. D. Aines and R. L. Newmark, Demolishing NAPLs, Civil Engineering, August, 1995a, 57-59.

Yow, J. L., Jr., R. D. Aines, R. L. Newmark, K. S. Udell and J. P. Ziagos, Dynamic Underground Stripping to remediate a deep hydrocarbon spill, Geoenvironment 2000: Characterization, containment, remediation, and performance in environmental geotechnics, Y. B. Acar and D. E. Daniel, eds., Proceeding of a specialty conference sponsored by the American Society of Civil Engineers, New Orleans, Louisiana, February 24-26, 1995b, 1306-1320.

Work performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract W-7405-Eng-48.

Table 1. Kolmogorov-Smirnov 2-sample test for fatty acid concentration distribution classified by sampling time (before- versus after-DUS treatment samples). The number of before- and after-DUS isolates analyzed was 52 and 50, respectively. This test compares the overall distribution of fatty acid concentration; the medians are presented for reference.

Fatty acid	Median % fatty acid		Test	
Variable	Before-DUS	After-DUS	Statistic	p value[1]
*i*15:0	0	17.1	2.49	<0.0001
*a*15:0	0	18.4	2.59	<0.0001
*i*16:0	0	5.1	2.30	<0.0001
*i*17:0	0.1	3.0	2.47	<0.0001
*a*17:0	0	4.2	2.78	<0.0001

[1]Kolmogorov-Smirnov significance level

Table 2. Degradation of benzene, toluene, or BTEX by heat-tolerant microorganisms. Microbial isolates were collected from sediments following electrical heating and steam remediation. Four purified cultures and a mixed culture were tested for BTEX degradation. Test conditions: ground water was amended with 5 mg/L benzene, 5 mg/L toluene, or 25 mg/L of BTEX; inoculated with 1 x 10^5 cells/mL and incubated at room temperature for 3 weeks. Substrate degradation was measured by gas chromatography and the results were calculated by dividing the compound concentration by the chemical control concentration; each datum was the average of three samples, each analyzed in triplicate.

	Substrate degradation (%)		
Microorganism	**BETX**	**Benzene**	**Toluene**
Rhodococcus	2	10	17
Streptococcus	8	11	29
Rhodotorula	32	45	54
Micrococcus	17	23	48
Mixed culture	20	19	51